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A Role for Airborne Particulates in High Mercury Levels of Some Cetaceans

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In a study of 25 *Tursiops truncatus* and two *Globicephala macrorhynchus* examined by necropsy, abundant HgSe was found in both the liver and in the respiratory system (lung and hilar lymph nodes). In the liver HgSe was consistently associated with the cell-breakdown pigment lipofuscin, whereas in lung and hilar lymph nodes it was consistently associated with particulates consisting of partially graphitic soot and silicates. This supports earlier suggestions that in the liver HgSe may be a storage end product of Hg metabolism, while adding the new suggestion that in the respiratory system HgSe may be inhaled, preformed in combustion emissions. © 1995 Academic Press, Inc.

INTRODUCTION

Worldwide pollution of oceans and other bodies of water by mercury (Hg) is in large measure attributable to the burning of fossil fuels and the incineration of solid wastes (KBN Engineering and Applied Sciences, Inc. 1992). The average Hg content of U.S. coal is 0.2 ppm, and a single large power plant can consume a million tons of coal a year (Schlesinger and Schultz, 1971). In the case of municipal solid waste, the Tampa, Florida area Hg emission by individual incinerators may be as high as 2400 kg/year (Hillsborough County Environmental Protection Commission, 1991). Although the greater part of nonhydrocarbon components of coal remains in the ash, Hg and selenium (Se) are almost completely volatilized (Van Hook, 1978).

Emission control equipment removes most of the particulates, reducing stack emissions by >90%. Of that which escapes into the atmosphere, much is soot, a mostly amorphous but partially graphitic form of carbon. Mercury and/or mercury selenide may adsorb onto soot in the smoke stack or in the smoke plume (Van Hook, 1978). Airborne soot particles could transport Hg into the marine environment either as nuclei for rain drop formation or by direct deposition on water.

The accepted explanation for Hg accumulation in animal tissue is through percutaneous absorption of Hg from an aqueous medium in lower life forms and ingestion of these forms by animals higher in the food chain. Protein-bound Hg finds its way via the circulatory system to the liver where it is found in the highest concentrations (Gaskin *et al.*, 1979).

In view of the acknowledged pollution of the air by Hg emission from incineration, it was the purpose of this study to see whether direct inhalation of particulates might be an alternative means of Hg acquisition.

The problem was approached in the following way: (a) Examination of sooty particulates in the respiratory system (lung and hilar lymph nodes) (Rawson *et al.*, 1991) for Hg and for silicates; (b) determination of the chemical form of Hg in the respiratory system; and (c) comparison of both the form and the associations of Hg found in the respiratory system with those found in the liver (Rawson *et al.*, 1993).

MATERIALS AND METHODS

Study subjects. To the nine Atlantic bottlenose dolphins (*Tursiops truncatus*) previously reported (Rawson *et al.*, 1991) were added 16 more animals whose lungs and hilar lymph nodes also contained particulate soot. Also studies were two short-finned pilot whales (*Globicephala macrorhynchus*), likewise exhibiting particulate soot. All these animals were stranded between Tampa Bay and Charlotte Harbor on the southwest coast of Florida between 1989 and 1992 and were brought to Mote Marine Laboratory for necropsy.

Tissue techniques. All tissues were fixed in 10% formalin, embedded in paraffin, cut at 5 μm, and mounted on glass slides. Both stained (H & E) and unstained sections were examined.

Chemical studies. Analyses for Hg were done by an acid-permanganate digestion, followed by cold-vapor atomic absorption using a Thermo–Jarrell–Ashe atomic absorption spectrophotometer.

Polarized light microscopy. This was performed by two of the coauthors (G.W.P. and E.M.H.) on deparaffinized 5-μm sections of *Tursiops* and *Globicephala* lung

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TABLE 1

Measured Lattice Spacings from Selected Area Electron Diffraction (SAED) Pattern (Fig. 2, Inset) Compared with Those of Mercury Selenide (HgSe, Tiemannite)

SAED pattern		HgSe [24]	
Spacing (Å)[a]	Intensity[b]	Spacing (Å)	Intensity (I/I_0)
3.36	vs	3.51	100
2.88	vw	3.04	16
2.06	s	2.15	50
1.75	s	1.84	30
1.69	vw	1.75	4
1.46	m	1.52	6
		1.40	10
1.34	m	1.36	2
		1.24	8
1.19	m	1.17	4
1.12	m	1.07	2
1.03	w	1.03	4
0.98	m	0.96	2
		(plus 6 other lines)	

[a] ±2.5% error.

[b] vw, very weak; w, weak; m, moderate; s, strong; vs, very strong.

or hilar lymph node stained with H & E (Patton *et al.*, 1992). A Zeiss Universal microscope equipped with plan achromat lenses, a polarizer, and an analyzer was used. Sections of tissue averaging 1 cm^2 were initially evaluated independently by both investigators at 125×. Sections were examined and particle loads were described as light, moderate, or heavy. In addition, particle distribution and birefringent color were recorded. Nonbirefringent carbon particle load was also observed. Intra- versus extracellular location of particles was determined at 500×.

Analytic electron microscopy. This was performed on a JEOL 4000FX, with 2-Å point-to-point resolution, equipped with a germanium detector for energy dispersive X-ray microanalysis. The Ge detector is capable of detecting X-rays as low in energy as those of oxygen. Selected area electron diffraction (SAED) was carried out to identify the HgSe crystals; calibration of the *d*-spacings was facilitated by the use of gold evaporated onto the amorphous carbon substrate of the TEM grid. Lattice fringe imaging of the HgSe and graphite confirmed the identities and helped to ascertain the relative amounts and disposition of these materials. Particle sizes of HgSe crystals were measured directly from the TEM negatives.

X-ray powder diffraction. This process employed paraffin-embedded tissue sections cut at 5 μm and deparaffinized with xylene. Black areas were picked out manually with a sharp tungsten needle, using a stereomicroscope at 12–24×. One to 5 ng was picked off at a time. This was continued until 0.5 to 1 μg of the black material was accumulated. Black specks were combined and attached with collodion to the tip of a fine glass fiber. The glass tip containing the sample (which usually consisted of about 50% black material, 25% tissue, and 25% collodion and glass) was exposed from 3 to 8 hr to CuKα X-rays in a 57-mm powder camera. The resulting lines were read visually.

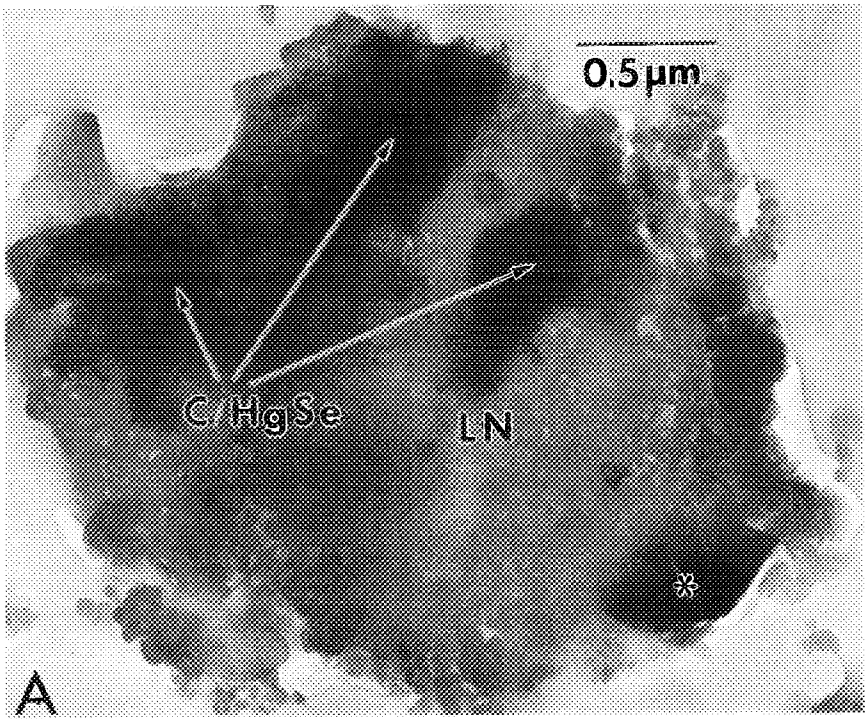
RESULTS

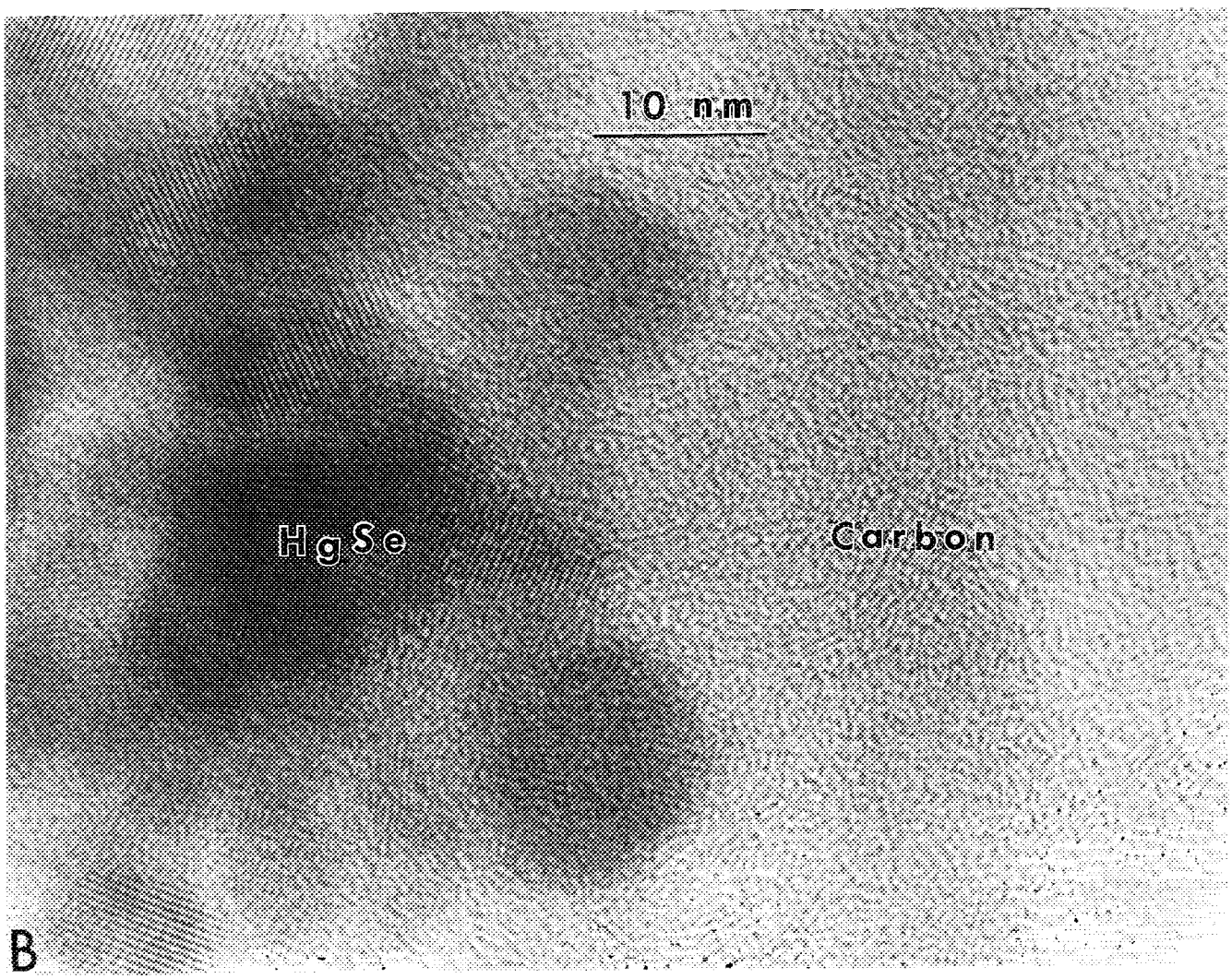
Examination of lung and hilar lymph nodes from both *T. truncatus* and *G. macrorhynchus* by polarized light microscopy revealed macrophages containing birefringent crystals and black deposits. Black deposits in hilar lymph nodes of both *T. truncatus* and *G. macrorhynchus* examined by analytical electron microscopy were identified as mostly amorphous but partially graphitic soot with mercury selenide (HgSe, tiemannite) (Table 1) adsorbed onto the soot in considerable quantity (Figs. 1A, 1B, and 2). The birefringent crystals proved to have a high concentration of silica as well as combinations of aluminum, potassium, calcium, phosphorus, sulfur, titanium, and iron, probably in the form of silicates including clay minerals. Many of these particulates (0.25–4 μm in diameter) in conjunction with soot lie within macrophages (Figs. 3A and 3B). Schmorl reaction for lipofuscin in these tissues was negative.

A previous study (Rawson *et al.*, 1993) of 18 livers of *T. truncatus* removed at necropsy exhibited the presence of dark pigment lying within the portal tracts in all animals having a Hg concentration greater than 60 mg/kg (wet wt). By analytical electron microscopy this pigment proved to be rich in HgSe. Schmorl reaction (Pearse, 1960) suggested that lipofuscin (residue to lysosomal digestion) was also a component of this material. Further study of the pigment by X-ray powder diffraction confirmed the predominant material to be HgSe.

In two particular individuals, a *T. truncatus* and a *G. macrorhynchus*, examination of the lymph nodes near the hilus of the liver revealed abundant dark pigment easily visible with the naked eye. By light microscopy the dark material more closely resembled the aggregated dark brown pigment seen in the liver portal tracts than it

FIG. 1. (A) Brightfield electron micrograph of lymph node (LN) tissue that was pressed between glass plates to electron transparency and mounted on a thin (~20-nm thick) carbon support substrate (10% neutral-buffered formalin, postfixed in 2.5% glutaraldehyde in phosphate buffer). A euhedral mineral grain, presumably an inhaled particle, is indicated. The dark regions are carbonaceous material and mercury selenide (HgSe). (B) High-resolution lattice fringe image of polycrystalline HgSe, with an average crystal size of 10–20 nm, and carbonaceous material. The poorly ordered turbostratic structure of the carbon is typical of combustion soots.


0.5 µm
C/HgSe
LN
A


10 nm
HgSe
Carbon
B

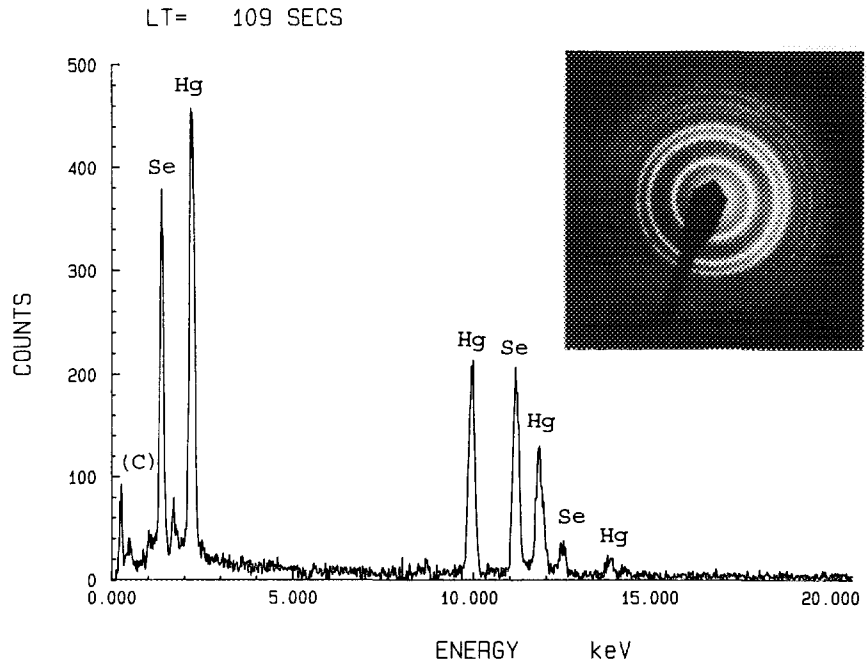


FIG. 2. Energy-dispersive X-ray spectrum from HgSe SAED pattern (upper right) was obtained from polycrystalline HgSe like that shown in Fig. 1A. The measured lattice spacings from this pattern are listed in Table 1.

did the particulate, greenish-black material noted in the lung hilar lymph nodes. The Schmorl reaction was positive with the pigment of the hepatic lymph nodes in contrast to the negative reaction in the pulmonary lymph nodes. Abundant HgSe was present in the dark material from the hepatic nodes of both animals. Both animals had very high concentrations of Hg in the liver tissue: the pilot whale 726 mg/kg and the dolphin 234 mg/kg (wet wt).

It is of interest to compare the dark material of liver, hilar lymph nodes, and hepatic lymph nodes with respect to both the HgSe crystals and the nature of the amorphous material. In both the liver and the hepatic lymph nodes, the HgSe crystals were small, averaging about 50 Å. HgSe crystals in the lung and hilar lymph nodes were much larger, measuring 250–500 Å. Abundant carbon was present in the hilar nodes, while only very small amounts were found in the hepatic nodes and the liver, with HgSe far more prevalent than carbon. Some of the liver Hg volatilized in the high-energy beam of the electron microscope while the pulmonary Hg resisted such volatilization.

DISCUSSION

These findings suggest two routes of entry for Hg into the cetaceans studied. Hg in the liver seems most likely to have been acquired trophically, passing through the gastrointestinal tract and being carried to the liver by way of the portal vein, and sequestered in the portal tracts. In the liver it may well have been processed and converted to HgSe, as suggested by Martoja and Berry (1980), accumulating as an end product. In animals producing large amounts of HgSe, some of this may be carried to the hepatic lymph nodes and even to the spleen, as appears in the case of the pilot whale. It has been noted that in the presence of Se, Hg can be transferred from organ to organ (Koeman *et al.*, 1973). By contrast, the HgSe, as well as silicates, in the lungs and hilar lymph nodes appears closely associated with carbon. This suggests an atmospheric association of these materials, possibly while still in a combustion emission stream, with subsequent inhalation and transport from the terminal airways by way of macrophages to the regional lymph nodes. The distinctly larger crystal size of HgSe in the respiratory system compared with HgSe in liver and hepatic lymph nodes adds further weight to the concept of different origins. However, *in vivo* accumulation or precipitation of HgSe onto the surfaces of inhaled soot particles cannot be absolutely ruled out.

Approximately 70% of the Se in the atmosphere is attributed to the burning of coal and oil and tends to aggregate as particulates (National Academy of Sciences, 1976), adding to the possibility that Hg and Se encounter each other and combine in the stack.

Methylmercury binds with sulfhydryl groups (Chang and Suber, 1982; Sumino *et al.*, 1977). Se binds methylmercury 100 times more tightly than sulfhydryl groups (Stillings *et al.*, 1974; Magos and Webb, 1980), and the strength of this bond suggests that HgSe may be removed from biological turnover, with subsequent accumulation (Kosta *et al.*, 1975) as a nonbiodegradable end product of Hg metabolism. This may, at least in part, explain the apparent tolerance of certain marine mammals for Hg.

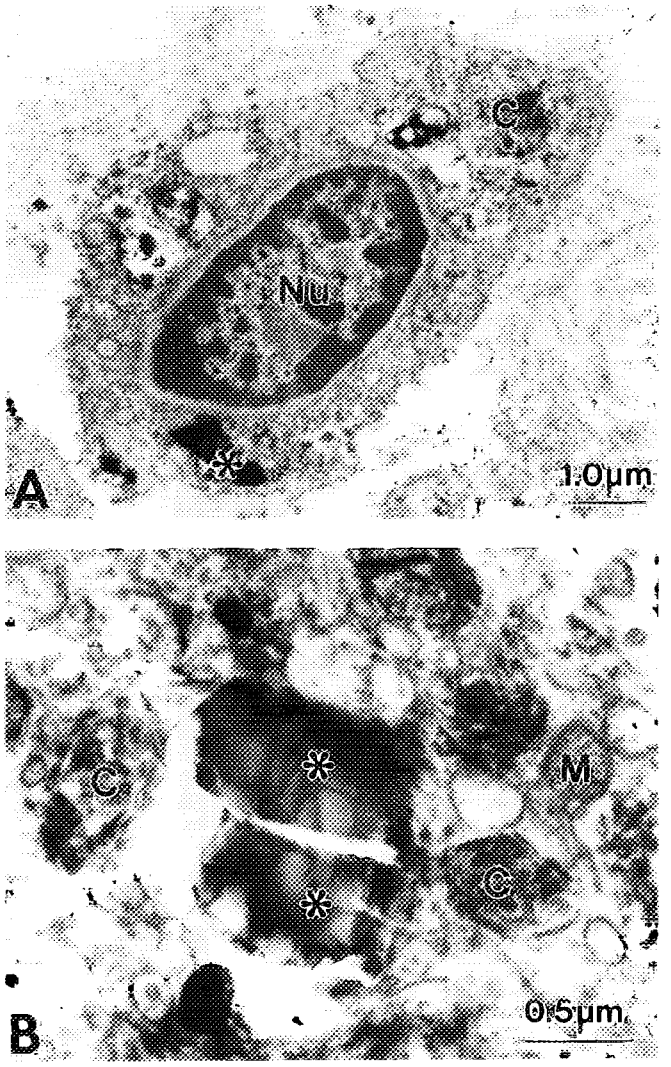


FIG. 3. (A) Degenerating cetacean macrophage containing various materials similar to air pollutants found in terrestrial animals and humans. (B) Representative crystalline material found in a macrophage from a dolphin that was stranded alive in the study area on Florida's central west coast.

Seal and dolphin livers are reported (Andre *et al.*, 1990) to commonly achieve 1:1 Se–Hg ratios, suggesting extensive storage as HgSe. An Environmental Studies Board Panel on Mercury (1978) found that the protective effect of Se is not documented in humans. The minimum calculated body burden of Hg to produce symptoms in humans is estimated to be 20 mg for a person weighing 70 kg (Nordberg, 1976). A calculation of the data for *T. truncatus* indicates that the minimum body burden to produce mild lesions, in this case mild fatty liver, is 600 mg for a 300-kg dolphin, about 7 times the human threshold. This agrees with the findings of Clarkson (1977) that the biological half-life of Hg in marine mammals is 10 times that in man and is compatible with the concept of HgSe as an end product.

CONCLUSION

In conclusion, this study suggests that in the liver of the marine mammals studied, HgSe is associated with cell-breakdown pigment and may be a stored end product of Hg metabolism, whereas in the respiratory system HgSe may be inhaled as preformed atmospheric particulates.

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